PROSTATIC CELLULAR LINE AND ITS USE FOR OBTAINING AN ESTABLISHED PROSTATIC TUMOUR IN AN ANIMAL

The present invention concerns a new established cellular line of dog prostatic cancerous epithelial cells, in an animal to which have been grafted cells from this cellular line generating an established prostatic tumour and to identification processes of therapeutic substances for the prevention and the treatment of the prostate cancer.

The prostate cancer in man is a rapidly growing pathology, and today constitutes at least 85,000 new cases per year in Europe. The current treatments of locally advanced and metastatic cancers are constituted by surgical or medical castration combined or not with antiandrogenic prescription; nevertheless, it is a palliative treatment because it becomes ineffective within an average time of 12 to 36 months, constituting the release phase of the hormonal treatment of the disease. In this phase of the release of the hormonal treatment, the other recognised therapeutics, chemotherapy, metabolic irradiation, etc., improve the quality of life of the patients but do not alter the fatal development of the disease.

Different techniques have been developed to follow the possible therapeutic effect of a treatment, such as described above, of prostatic tumours. This follow up consists essentially of measuring the specific antigens level of the epithelial cells of the prostrate in the blood. When the level of these antigens increases, it can be the reflection of an abnormal increase of the number of prostatic epithelial cells, a sign of a tumorous progression. Amongst these antigens, the PSA (for prostate specific antigen) is the most used antigen tracer. It is a member of the family of kalikreins.

caused after grafting in the prostate of the aforesaid animal of 10^7 to 10^9 cells of an established cellular line, obtained after putting into culture and mechanical separation of cells of a spontaneous prostatic tumour existing in an animal B of the same species or of a different species.

In order that in line established cells in a prostate of an animal take in the best conditions, it is preferable that the animal from which the prostatic tumorous cells are removed and the receiving animal are of the same species. Taking account of what has been said above, between the etiological similarity of prostatic tumours in the dog and in the man, the choice of the dog as the animal to establish a prostatic tumour model enabling treatment products and methods to be tested appears as particularly appropriate.

In the invention's process, the grafting in the animal's prostate of previously established in line tumorous cells must be permanent, in other words not to risk undergoing a graft reject. To that end, the animal is treated by an immunosuppressive drug, as for example cyclosporin, simultaneously with or before the grafting of the aforesaid line cells. When the cyclosporin is used, it is administered to the animal in a dose of between 1 and 10 mg per kilo and per day. The immunosuppressive drug preferably begins at least two days before the grafting of the aforesaid cells, and preferably at least five days.

The present invention also concerns an established cellular line obtained after separation and putting into culture of cells of a spontaneous prostatic tumour existing in an animal, the cells of the aforesaid line being able to be grafted in the prostate of an animal of the same species or of a different species, and bearing essential

characteristics of the human prostatic tumorous epithelial cells.

For the reasons explained above, it is preferable that the donor animal, i.e. from which the prostatic cells are removed and established in line, and the receiving animal are of the same species; preferably this species is the dog so as to constitute an animal model useable in pre-clinical tests. By useable, is understood the reliability of the potential transposition into man.

The cellular line is established by removing an established prostatic tumour in a dog, mechanical separation of the tumour and putting into culture in flasks containing an appropriate nutritive environment. After propagation of the culture in this environment, the cells are treated with trypsin/EDTA. After a certain number of passages, the cells are then progressively adapted to the culture in the same nutritive environment.

In the invention, every particular attention has been concerned with the characteristics of the established line in culture, as well as the prostatic tumour obtained after grafting of the cells of the line in the dog's normal prostate.

The essential characteristics of an established line in accordance with the invention and obtained after separation of a dog's prostatic tumour then put in culture are on the one hand, that the karyotype is not less than 60 chromosomes, and, on the other hand, that the doubling time, between 20 and 35 hours is not modified by the presence of dihydrotestosterone whatever is the concentration of this latter. In addition, the line in accordance with the invention does not form agar colonies.

The cellular lines in accordance with the invention and the prostatic tumours obtained after grafting of 10^7 to 10^9 cells of the aforesaid line have common cytological and histochemical characteristics characterising the prostatic epithelial cells' cancer.

a) The first important characteristic is the recognition of tumorous cells by a human anti-PSMA monoclonal antibody. The PSMA or specific membranous antigen of the prostrate is a new tracer expressed by the epithelial cells of the normal, hyperplastic or cancerous prostrate. The PSMA is a transmembranous glycoprotein of which almost 95 % is situated outside the cell. This protein has been discovered thanks to a monoclonal antibody, called 7E11-C5.3, produced by Horoszewicz et al (Anticancer Research, 1987, 7: 927-936) against a membranous preparation coming from the cancerous prostatic line LNCaP. L'AND complementary to the PSMA has been cloned by Israeli et al (Cancer Research. 1993, 53: 227-230) which has enabled its primary structure to be deduced in amino acids. The PSMA is constituted from 750 amino acids of which the 19 N-terminals are intracellular, the following 24 transmembranous and the remaining 707 extra cellular. The potential interest of this new prostatic tracer is that it appears over expressed in the prostatic cancer and more particularly in the not very dissociated and metastatic carcinomas as well as in the prostatic cancerous cells after an androgeno-suppressive therapeutic (Wright et al., Urological Oncology, 1995, 1: 18-28). The existence of a human anti-PSMA monoclonal antibody recognising the human PSMA and also recognising the canine PSMA enables the identification and the follow up of the development of the cancerous cells which constitutes a quality insurance of the animal model in accordance with the invention. Indeed, the more the animal model resembles

the specific biological elements of the prostate common with man, the more the extrapolation of the obtained results will be reliable.

The present invention also concerns a human anti-PSMA monoclonal antibody, called PSM-P12 and registered with the CNCM on the 6 August 1999 under the number I-2280. This antibody has been produced against a peptide corresponding to the amino acids 44 to 62 (lys - ser - ser - asn - glu - ala - thr - asn - ile - thr -pro - lys - his - asn - met - lys - ala - phe - leu) and localised in the N-terminal part of the extra cellular structure of the PSMA. It has been selected for its capacity to trace the normal human prostatic epithelial cells by immuno-histochemistry. This antibody specifically recognises the canine prostatic cancerous cells of the animal model. It also recognises the cells of the human prostatic line LNCaP described by Horoszewicz et coll. (Cancer Research , 1983, 43: 1809-1818).On the other hand, this antibody does not recognise the cells of human lines not expressing the PSMA like DU-145 described by Stone et coll. (International Journal of Cancer, 1978, 21: 274-281). On the other hand, a cellular line such as the line PC-3 described by Kaighn et coll. (Investigations in Urology, 1979, 17: 16-23) expresses a PSM membranous antigen having a partial homology with the PSMA of the LNCaP line, is partially recognised by the anti-PSMA antibodies produced against the same PSMA N-terminal part of the extra cellular part of the antigen.

All monoclonal antibodies having the same PSMA eptitoptic recognition characteristics must be considered as a functional equivalent of that.

b) the established cellular lines in accordance with the invention and the prostatic tumours stemming from the grafting of the line in a canine prostate have also as common characteristic to be recognised by antibodies

The present invention also concerns a non human mammal animal bearing a prostatic tumour likely to be obtained after grafting on the prostate of the said animal of 10^7 to 10^9 cells of an established cellular line after putting into culture of a prostatic tumour. Preferably coming from the same animal species, mechanically separated then trypsinized after several passages in a nutritive environment. The preferred species in question is the dog. The prostatic tumour caused in this animal in accordance with the invention has the same characteristics as that of the cellular line in accordance with the invention. These characteristics are common to a dog prostatic tumour and to a human prostatic tumour. The animal in accordance with the invention, and preferably the dog, therefore constitutes an excellent laboratory model reproducing the characteristics of the human prostatic tumour and therefore in fact a tool of choice in the pre-clinical experimentations of substances liable to treat the prostatic cancer in man and in the dog.

It is also one of the objects of the present invention to supply a method for identifying a substance susceptible to treating a prostate tumour, the aforesaid method including administering the effective doses of the aforesaid substance to an animal and the detection and the measurement by comparison with a substance not suspected of having a therapeutic effect of an effect on a reduction of the aforesaid tumour.

The animal in question in this method is a carrier animal of a prostatic tumour, itself developed by grafting of cells of a previously established line, the aforesaid line coming from the setting in culture of a previously developed prostatic tumour in an animal preferably of the same species as the animal to which cells are grafted. In a preferred way, the animal in question is the dog taking into account the similarities of the phenotypic and

histological characters of the prostatic tumours in the dog and the man.

By effective does, it is understood, as in any screening method, the administration of a dose likely to have a preventive or curative effect on the development of a cancer of the prostate.

By substance, is understood any substance of potential therapeutic interest which is for example an organic substance based on different basic chemical backbones or of biological macromolecules having an effect of repression or inhibition of the expression of specific genes of the cancer of the prostate; that can be lastly a vector or a viral particle carrier of a suitable sequence of interest for the genic therapy of this type of cancer.

By "specific gene of the prostate", is understood here a gene whose expression is limited to the prostate cells and more particularly to its epithelial cells, and whose expression is generally undetectable in normal cells derived from other tissues than those of the prostate. In a general way, and as the knowledge of the etiology of the development of the cancer of the prostate makes it possible to make the assumption that such or such candidate could allow to make regress a tumour or transform a tumorous cell into normal cell. The method in accordance with the invention which makes use of an animal model representative of that which could occur in the man would be able therefore to be used in pre-clinical tests.

The effect of the effect of a substance on the tumour can be measured by any known means available to the skilled worker. It can be for example immuno-imaging or histological examinations of a biopsy of the tumour. The immuno-imaging has the advantage of enabling the use of a specific monoclonal antibodies range or other antigen,

Briefly, the cells are fixed with a methanol/acetone (2/1) mixture for 15 min. After three washes in PBS, in the presence of 0.1 % BSA, the immunofluorescence is achieved by incubation at ambient temperature for 1 hour with the appropriate dilution of monoclonal or polyclonal antibodies. The cells are then incubated with the second antibody traced with the fluorescent. For each test, a negative control was carried out by using monoclonal or polyclonal antibodies having no chance of having an affinity for the cellular antigens but of the same sub-class of immunoglobulin.

Antibodies used

The antibodies used are shown in table 1 below.

Parameters	Ac Type	Results
Cytokeratine 8	M	Neg
Cytokeratine 14	M	Pos
Cytokeratine 18	M	Neg
Cytokeratine 19	M	Pos
Vimentine	M	Pos
Ki67	M	Pos
PSA	P	Pos
PAP	M	Neg
CGA	P	Neg
NSE	M	Neg
Androgen receptor	M	Neg
EGF receptor	M	Neg
FGF receptor	M	Neg
PSMA (PSM-P12)	M	Pos

In the central column, the letter "M" indicates that it is a monoclonal antibody and the letter "P" of a polyclonal antibody. In the third column, the term "Pos" indicates the existence of a positive reaction between the monoclonal or polyclonal antibody on the canine established line.

The immunofluorescence results obtained from the biopsy of this established canine cancer indicate the same reactivities with the monoclonal antibodies shown in table 1 above as the reactivities obtained with the DPC-1 line, and which show the characteristics of a human prostatic cancer.

Example 3: Tumorigenicity of the DPC-1 line

Besides the grafting of the line in a canine prostate and the results described in example 2 above, the cells were injected in the feet pad of a nude mouse. The formation of a tumour in the lymphatic ganglions was observed in 100 % of the cases after three to six weeks following the injection.

Example 4: specificity of the PSMA-P12 monoclonal antibody

We recall that this monoclonal antibody was produced against a peptide of 20 amino acids localised in the extra cellular structure of the PSMA. It was selected for its capacities to trace the normal human prostatic epithelial cells by immuno histochemistry. Figures 2a and 2b indicate respectively the specificity of the anti PSMA P12, as much on the DPC-1 canine line as the LNCaP human line, which as such is a significant argument in favour of the validity of this line as a human tumorous prostatic line model. Figure 6 shows that this antibody is specific as much on the human prostate cancers as on the canine prostate cancers, which also indicates that the animal model carrying the tumour established after grafting of the DPC-1 line cells is a model for which the tumour faithfully reflects the characteristics of a human tumour. It has indeed been observed that this antibody has the same specificity for the canine prostate (figure 6) as for the human prostate (figure 7).